THE CONSTITUTION OF THE ALKALOID CARPAINE.

By

T.S. WORK, B.Sc.

Thesis presented for the Degree of Ph.D.,
University of Edinburgh.

May 1936.

Contents.

I. GENERAL and THEORETICAL.

Carpaine was discovered by Greshoff in the leaves of the Papaw tree (Carica papaya, L.). The formula $C_{14}H_{27}O_2N$ was assigned to it by Merck (Jahresber., 1891, p. 30), but this was later corrected to $C_{14}H_{25}O_2N$ by van Ryn (Inaug. Diss., Marburg, 1892; Arch. der Pharm., 231, 184, 1893; 235, 332, 1897).

Van Ryn gives a detailed description of the plant which he says contains carpaine in appreciable quantity in the leaves only. Details are also given for different methods of extraction of the alkaloid and for the preparation of a number of salts. According to Greshoff, young leaves contain as much as 0.25% carpaine and adult leaves up to 0.07%. Later Barger (J.C.S., 97, 466, 1910) obtained 0.07% from adult leaves, but Barger, Girardet and Robinson (Helv. Chim. Acta, 16, 90, 1933) found that leaves from specially grown seedlings contained only 0.022% and young leaves from adult trees 0.036%. They accordingly suggest that "there appears to be an optimum age of the leaves". This is borne out by the results of the present investigation/

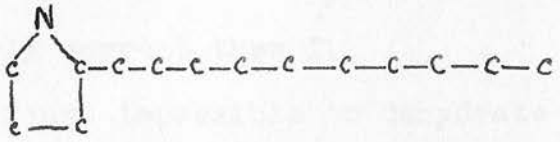
investigation where old leaves from British India gave a yield of only 0.018%. Van Ryn also attempted to obtain an insight into the constitution of carpaine by hydrolysis and by permanganate oxidation but without success. Methylation of carpaine by means of methyl and ethyl iodides is also described but according to van Ryn treatment of ethyl carpaine ethiodide with silver oxide did not give a quaternary base (cf. p. 41).

The first evidence of the structure of carpaine was obtained by Barger (J.C.S., 97, 466, 1910). In this paper it was shown that carpaine is the internal anhydride of a substance possessing both acid and basic properties which is produced on boiling carpaine with strong acid. This substance contains a carboxyl group and has the composition $C_{14}H_{27}O_3N$; the name carpamic acid was suggested for this compound. It was shown that both carpaine and carpamic acid ethyl ester yield nitroso derivatives, so that the internal anhydride must be a lactone and not a lactam. Further it was shown that on oxidation of carpamic acid by nitric acid (and possibly also on oxidation with potassium permanganate) a dibasic acid/

acid was obtained of the composition $C_8H_{14}O_4$ which was suggested to be dimethyl adipic acid.

A further paper by Barger, Girardet and Robinson (Helv. Chim. Acta, 16, 90, 1933) extended these observations. The fatty acid obtained on oxidation with nitric acid was identified as suberic acid. A second fatty acid was also isolated as an oxidation product and shown to be azelaic acid $C_9H_{16}O_4$. The presence of a pyrrolidine ring was demonstrated by dehydration with selenium, when a pyrrole derivative was obtained. It was suggested that the long carbon chain from which the two fatty acids arise was attached in the α-position to the nitrogen, and it was pointed out that it was tempting to assume some relationship to a naturally occurring fatty acid, and to place all the carbon atoms in an unbranched chain.

The skeleton formula for carpamic acid on this basis would be:

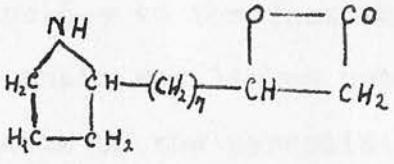


It was further shown that carpamic acid is formed from/

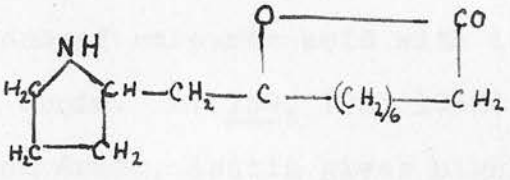
from carpaine, not only by the action of acid but also, readily, by the action of alkali in alcoholic solution, thus supplying further evidence of the lactone structure. It appears to be impossible however to regenerate the lactone from the acid by the ordinary method so that carpaine is probably not a γ or δ lactone.

On the basis of this evidence Barger, Girardet and Robinson (Helv. Chim. Acta, 16, 90, 1933) suggested two possible formulae:

I.



II.



The further evidence obtained during the course of the present investigation seems to indicate that II is more nearly correct than I.

It was found impossible to dehydrate carpamic acid either by zinc chloride or by acetic-sulphuric acid mixture. The oxidation of the secondary hydroxyl/

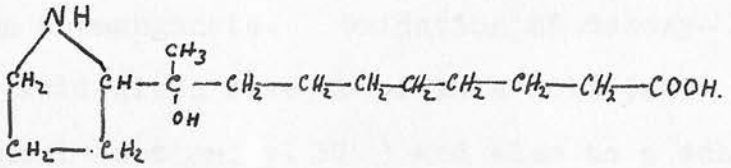
hydroxyl group, which should be present in carpamic acid if the above formula is correct, was therefore attempted. No evidence of the formation of a keto acid was obtainable so that the existence of a tertiary and not a secondary hydroxyl was suggested. The determination of side chain methyl ($C-CH_3$) by the Kuhn-Roth chromic acid method on a sample of carpamic acid gave results indicating the presence of a single C-methyl (see p.21).

There was the possibility, however, that this result might be due to the fact that in carpaine the long side chain was linked not to the α but to the β carbon atom of the pyrrolidine ring. Evidence that this is not the case was provided by the colour reactions of carpamic acid with isatin (Grassman and Arnim. A. 159, 192, 1935). According to Grassman and Arnim, isatin gives highly coloured complexes with any pyrrolidine compounds in which carbon atoms 2 and 5 are unsubstituted. Carpamic acid gives no such coloured complexes, so that the long side chain is evidently in the α position to the N of the pyrrolidine ring. The suggestion is then put forward that the C-methyl is a branch of the/

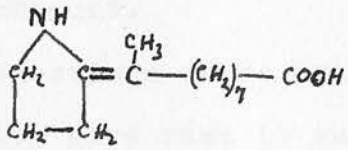
the long side chain, and attached to the same carbon atom as the alcoholic hydroxyl. The existence of this C-methyl branch was further confirmed by examination of the hydrocarbon obtained from carpamic acid by reduction with red phosphorus and hydriodic acid. This hydrocarbon was found to have one C-methyl according to the Kuhn-Roth chromic acid method (see p. 33).

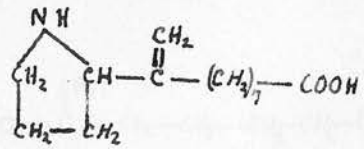
It was found that on chlorinating carpamic acid hydrochloride (see Experimental Section, pp.26-28) and removing the chlorine from the resulting chloro compound by means of strong alkali an unsaturated product was obtained, presumably with the double bond attached to the C atom to which the hydroxyl group was previously attached. For this substance the name desoxy-carpamic acid is proposed. It was not isolated in the pure state itself but was characterised by catalytic reduction to the corresponding fully saturated compound dihydrodesoxy carpamic acid, which was readily purified (Experiment 12). Further evidence/

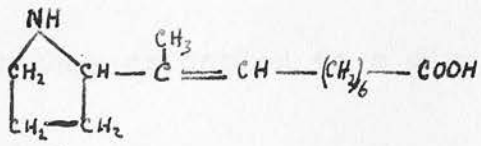
evidence on the structure was obtained on oxidation of desoxy carpamic acid by means of potassium permanganate (Experiment 17). The following formula (III) may be used to represent carpamic acid in accordance with the evidence already advanced, and used as a basis of discussion.

III. 

Assuming formula III for carpamic acid, there are three possible positions on the double bond in desoxy carpamic acid, so that it may be represented by one of the three formulae IV, V, VI.

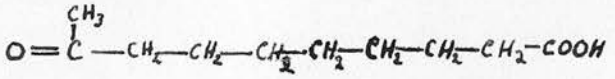
IV. 

V. 

VI. 

Formula V appears to be improbable on general grounds, but it is more difficult to decide between IV and VI. It is not really necessary to distinguish between them for our purpose however, it being sufficient to show that one of the two formulae explains the experimental results obtained on the oxidation of desoxy carpamic acid by potassium permanganate. Oxidation of desoxy-carpamic acid gives rise to azelaic acid (see Experimental Section, p. 37) and also to a substance which gave the pyrrole pine splint reaction on treatment with ammonia and subsequent distillation with zinc dust (Experimental Section, p. 38). It was not fully characterised but is evidently either succinic acid itself in an impure state, or a closely related compound.

The substance represented by formula IV should on oxidation give rise to succinic acid and a keto acid, ν keto decoic acid (VII).

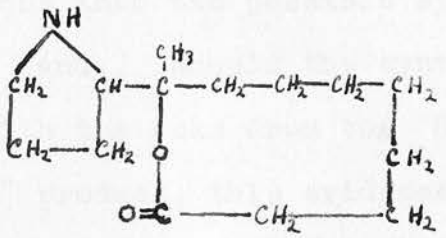


VII.

which on further oxidation to a dicarboxylic acid would/

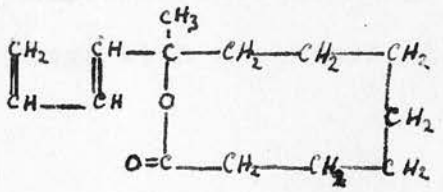
would probably give under the conditions of the experiment(slightly alkaline permanganate), a mixture of azelaic and suberic acids. Suberic acid was not isolated but indications of its presence were obtained (see Experimental Section, p. 38). The experimental evidence therefore favours formula IV for desoxy carpamic acid, and is also therefore in favour of the formula advanced for carpamic acid(III).

In an attempt to confirm this structure the exhaustive methylation of carpaine was studied. Carpaine according to the theories already advanced will have the structure VIII.



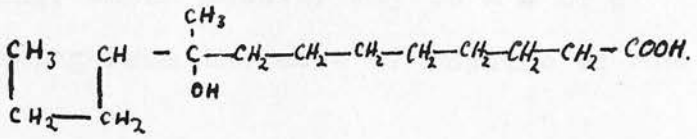
VIII.

so that the exhaustive methylation should yield a nitrogen free lactone of the structure IX.



IX.

It was found that on exhaustive methylation a neutral unsaturated compound was obtained in small yield. This compound on catalytic reduction took up the expected quantity of hydrogen and on hydrolysis gave rise to a fatty acid which could be distilled in vacuo and gave a well defined crystalline p-phenylphenacyl ester. If the structural formula (VII) for carpaine is correct, this compound will be the p.phenylphenacyl ester of ω methyl ω oxy-tridecylic acid (X).

X. 

Investigations into the possible synthesis of the acid are in hand. Should the synthetic acid be identical with the acid from the "Hoffmann degradation" product, this evidence along with that already obtained, will definitely establish the constitution of carpaine.

Carpaine would appear then to be the first alkaloid studied in detail in the structure of which a many membered lactone ring occurs. That carbocyclic rings with more than eight members occur/

occur in nature was first shown by Ruzicka (Helv. Chim. Acta. 1926, 9, 230). It was later shown by Kerschbaum (Ber., 1927, 60, [B], 902) that many membered lactone rings occur in certain plant oils. Numerous alkaloids are known with γ or δ lactone ring structures such as the narcotine-papaverine group, the pilocarpine group, hydrastine and dioscorine, while recently H. Schild (Ber., 69, 74, 1936) has studied the alkaloid obtained from Stemona.Sessilifolia and has shown the presence of a lactone ring, which however may be a γ or δ lactone, judging from the ease of rupture of the ring.

According to van Ryn, carpaine acts physiologically as a heart poison with a somewhat similar action to digitalis. On hydrolysis of the lactone group the characteristic bitter taste is lost and the digitalis-like action on the heart disappears. The lactone ring structure in carpaine would therefore appear to be connected with the characteristic heart action and the bitter taste. In this respect it may be compared with dioscorine (Gorter, Rec. Trav. Chim. , 1911, 30, 161) which has a very bitter/

bitter taste and which produces paralysis of the central nervous system and in general behaves like picrotoxin. This action is suppressed on hydrolysis of the lactone ring.

As regards the formation of carpaine in the plant, it is to be expected that the pyrrolidine ring is formed in the same manner as in the other alkaloids of the pyrrolidine group such as hygrine, cuschygrine, the tropane alkaloids, nicotine, etc.

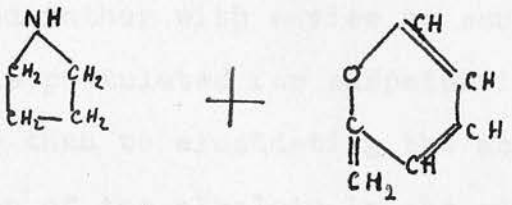
The suggestion of Barger, Robinson and Girardet that carpaine is related to the fatty acids seems rather unlikely on the basis of the new formula, firstly because the alkaloid has not a straight chain of 14 carbon atoms but a branched chain with 13 carbon atoms in line (an odd number of carbon atoms), and secondly that if formed from a naturally occurring fatty acid one would expect the side chain methyl group to be in the centre of the chain. Admittedly these objections might be overcome, but if the theory of Robinson (J.C.S., 1917, 111, 876) for the formation of the pyrrolidine ring from ornithine and formaldehyde in other alkaloids/

alkaloids is to be accepted, it seems unnecessary to postulate a different method of formation of the ring in carpaine because there is a long carbon chain in the molecule.

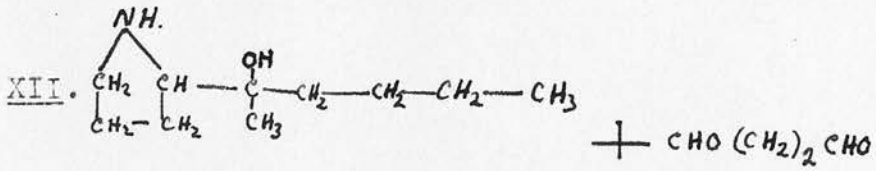
It is possible, however, to devise a natural synthesis of carpaine starting with glucose, ornithine and formaldehyde, naturally occurring plant products, and using only such reactions as are generally believed to occur under natural conditions, such as oxidation, reduction and aldol condensation.

Ornithine and formaldehyde according to Robinson might yield succindialdehyde and methylamine, these then condensing to give dihydroxy-n-methyl pyrrolidine which is a potential source of a fully saturated pyrrolidine ring. On condensation of the hypothetical unsaturated substance (XI) derived from glucose (cf. Robinson, Proc. 9th International Congress of Pure and Applied Chem. 1934, 5, 23) and subsequent reduction of the double bonds the substance (XII) might be produced, and this compound on further condensation with succindialdehyde or acetone dicarboxylic acid could lead to carpamic/

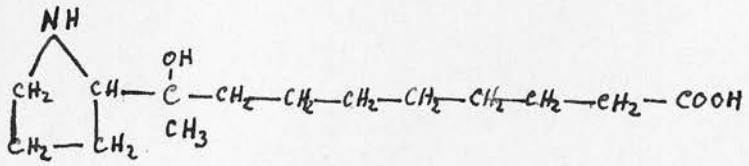
carpamic acid. The formation of carpaine from carpamic acid although not effected in the laboratory so far, is presumably a reversible process.



XI.



XII.



CARPAINE

Admittedly this scheme may appear somewhat artificial, but owing to the rather unique structure of carpaine a more straightforward scheme is not readily devised. This scheme is suggested rather with a view to showing that the structure postulated for carpaine is physiologically possible than to elucidating the actual mode of formation of the alkaloid in the plant.

II. EXPERIMENTAL.

EXPERIMENTAL.

Page

EXPERIMENTAL.

1. Extraction of the alkaloid Carpaine from the leaves of Carica papaya.

100 kilos of powdered leaves were percolated with 80% alcohol containing 0.5% acetic acid. The alcohol was recovered in vacuo and the extract was worked up by the following process.

The extract was diluted with ether and shaken up in portions in a separating funnel with 2% hydrochloric acid. The acid was run off and the process was repeated until the acid extract gave only a feeble 'Mayer reaction'. The combined acid extracts were shaken up with fresh ether until all the chlorophyll was removed. The aqueous extract was then made alkaline with ammonia and the alkaloid extracted with ether.

It was found that the alkaloid could most easily be obtained from this extract in a crystalline state by allowing the ether to evaporate slowly at room temperature, when the crude carpaine separated in/

in large crystals. Two recrystallisations from methyl-ethyl-ketone gave a pure product.

From 100 kilos of leaves were obtained 18 gm. of carpaine.

2. Preparation of Carpamic Acid.

2 Gm. of carpaine were added to 30 c.c. of concentrated hydrochloric acid in a flask fitted with a reflux condenser and a ground glass joint and boiled 8-10 hours. The hydrochloric acid was removed in vacuo and the carpamic acid hydrochloride recrystallised from acetone. 1.95 Gm. of a fine white crystalline powder, m.p. 161°, were obtained, readily soluble in water and in alcohol, but almost insoluble in cold acetone.

The carpamic acid hydrochloride was treated with freshly precipitated silver oxide and the aqueous filtrate evaporated to dryness. The residue was recrystallised from alcohol and finally sublimed in the high vacuum at 210-220°. The long white needle crystals so obtained had a m.p. of 224°, were readily soluble in water, sparingly soluble/

soluble in cold alcohol and insoluble in acetone and other organic solvents.

C-methyl analysis.

A determination of side chain methyl ($C\text{-}CH_3$) by the method of Kuhn (chromic acid oxidation) gave the following results.

Mg. substance	c.c. N/100 NaOH	$C\text{-}CH_3$
16.155	6.23	0.99
20.550	8.01	1.00

3. Dehydration of Carpamic Acid.

(a) The action of zinc chloride and acetic acid on carpamic acid.

0.1 Gm. carpamic acid, 0.1 gm. zinc chloride and 3 c.c. of glacial acetic acid were boiled under a reflux condenser for 2 hours. Acetic acid was removed in vacuo and zinc by treating with ammonia, evaporating to dryness and extracting with alcohol. The resulting oil could not be crystallised.

(b) /

(b) The action of acetic-sulphuric acid mixture on carpamic acid.

0.4 Gm. of carpamic acid was treated with 10 c.c. of a mixture of two parts concentrated sulphuric acid to one part glacial acetic acid (specially prepared by distillation from a H_2SO_4-HAc mixture). The mixture was heated for 10 hours at 170° under a reflux condenser. Considerable charring was observed. Sulphuric acid was removed as the sulphate and the residual material found to consist almost entirely of unchanged carpamic acid.

4. Oxidation of Carpamic Acid by Chromic Acid.

0.8 Gm. of carpamic acid hydrochloride was dissolved in water and the calculated quantity of chromic acid mixture added (Beckmann mixture for the oxidation of a secondary OH to a ketone). The mixture was heated on the steam bath for one hour and then tested for free dichromate with hydrogen peroxide. A negative result was obtained. The mixture was diluted with water and made alkaline with ammonia, the precipitated chromic hydroxide being filtered off. The filtrate was evaporated to/

to dryness in vacuo and extracted with alcohol. The evaporation and extraction were repeated until all the inorganic material was removed and the oil finally obtained was then dissolved in the minimum quantity of hydrochloric acid. On evaporation of the water, a semi-crystalline mass was obtained which was dissolved in hot acetone and from which on cooling crystals of carpamic acid hydrochloride separated. m.p. 157-158°. Mixed m.p. with authentic specimen 160°. 40% of the starting material was recovered unchanged. The residual oil which was insoluble in acetone but soluble in methyl alcohol could not be crystallised and did not give reactions for a ketone.

5. Preparation of Diacetyl Carpamic Ester.

1 Gm. of carpamic acid hydrochloride was dissolved in 10 c.c. of dry methyl alcohol and dry hydrochloric acid gas passed into the mixture. The resulting methyl ester was acetylated by treatment with a mixture of 10 c.c. of acetic anhydride, 2 gm. of sodium acetate and 5 drops of pyridine, the whole being heated on the water bath for one hour. The/

The acetic anhydride was removed in vacuo and the residual oil distilled in a high vacuum with the metal bath at a temperature of 250-270°. Yield 0.9 gm. (crude).

6. Action of thionyl chloride on N acetyl carpamic acid.

0.9 Gm. of diacetyl carpamic ester was treated with N/10 NaOH in order to remove the O-acetyl group and the resulting N acetyl carpamic acid distilled (with decomposition) under a high vacuum. The distillate was dissolved in chloroform and treated with excess of thionyl chloride with warming on the water bath. The excess of thionyl chloride was removed in vacuo, and the resulting brown oil treated with water to decompose any acid chloride which might have formed, and the product shaken out into ether from acid solution. On distillation a pale yellow oil was obtained which did not crystallise. The oil contained organic halogen.

7. /

7. Preparation of N-methyl Carpamic Ester.

1.3 Gm. of carpamic acid hydrochloride were heated in a sealed tube with 1.3 c.c. of 40% formaldehyde and 2.6 c.c. of water at 120-130° for 4 hours. The residue after removal of water was dissolved in ethyl alcohol and esterified by passing in dry HCl gas. The resulting mixture was diluted with water and extracted with ether, the ether extract being washed with dilute sodium carbonate. The resulting oil after removal of ether was distilled in the high vacuum at 190-195°. Yield 1.15 gm.

8. Chlorination of N-methylethyl Carpamate.

0.5 Gm. of N methyl carpamic ester was dissolved in ether and the theoretical quantity of thionyl chloride added. The mixture was allowed to stand in the ice-chest overnight. It was then washed with dilute aqueous sodium carbonate and with water. The brownish oil obtained on evaporating the ether was distilled in a high vacuum with the bath at 215°, the resulting clear oil crystallising on standing/

standing for several days. Yield 0.2 gm. A sample submitted to analysis gave the following results:

4.233 mg. subst. gave 2.055 mg. AgCl = 12.01% Cl.

Calculated for $C_{17}H_{32}NClO_2$, N methyl chloro carpamic ester, 11.2% Cl.

9. Chlorination of Carpamic Acid Hydrochloride.

Rather more than the equivalent quantity of thionyl chloride was added to 0.5 gm. of carpamic acid hydrochloride and the mixture allowed to stand in the cold for some time. The excess of thionyl chloride was destroyed by the addition of water. The brownish mass obtained on removing the water was found to decompose on distillation and could not be readily crystallised. It was therefore used for the next experiment without further purification on the assumption that it was at least partly mono chloro carpamic acid. This assumption is justified by the result of the previous experiment where, under similar conditions, N-methyl/

N-methyl carpamic acid ethyl ester was found to give a stable mono chloro derivative.

10. Action of Silver Oxide on Chloro Carpamic Acid Hydrochloride.

0.3 Gm. of crude chloro carpamic acid hydrochloride from the previous experiment was warmed on a water bath for one hour with excess of freshly precipitated silver oxide; the mixture was filtered hot and on cooling set to a stiff gel (silver salt). Silver was removed as the sulphide by means of H_2S. The aqueous filtrate was evaporated to dryness when a white semi-crystalline mass was obtained, soluble in water and in alcohol, but insoluble in the other organic solvents. This substance could not be purified by recrystallisation. The substance decolorised acid or alkaline permanganate almost instantaneously, whereas carpamic acid hydrochloride decolorises permanganate relatively slowly. The presence of a double bond is therefore indicated.

11. /

11. Catalytic Reduction of the Double Bond in Desoxy Carpamic Acid.

0.2 Gm. of impure desoxy carpamic acid from the previous experiment was dissolved in acetic acid and reduction attempted by means of hydrogen and platinum (Adams catalyst). It was found that the trace of sulphur present (from the H_2S used in the previous experiment) poisoned the catalyst so that only a small volume of hydrogen was taken up. On adding further portions of catalyst there was further adsorption of hydrogen. The solution was filtered and evaporated to dryness, the residue being sublimed under a high vacuum. Two distinct fractions were obtained, one subliming from 165-175°, and the other from 200-220°. These were resublimed repeatedly until constant melting points were obtained.

Substance 1 sublimes at 165-175°, m.p. 180-182°.

Substance 2 gave with carpamic acid (m.p. 224°) a mixed m.p. of 220-223°, and is evidently unchanged carpamic acid.

Substance/

Substance 1 was in too small quantity for further examination but is presumably dihydrodesoxy carpamic acid.

Various experimental modifications were tried in order to increase the yield, the method described in the next experiment being found the most convenient.

12. Preparation of dihydrodesoxy carpamic acid.

The following method was found to give the best yields. 1 Gm. of carpamic acid was dissolved in the minimum quantity of phosphorus oxychloride and phosphorus pentachloride was added until no further reaction was observable. The mixture was warmed on the water bath for 2 hours and the oxychloride then removed in vacuo. The residue was boiled with 25% methyl alcoholic potash for 4 hours, hydrochloric acid added until the solution was just acid to methyl red, and the mixture evaporated to dryness. Inorganic salts were removed by repeated extraction with alcohol and evaporation to dryness. Finally a clear oil was obtained which could not be crystallised, was soluble in alcohol and water, but/

but insoluble in acetone and ether. This oil was hydrogenated using Adam's catalyst in acetic acid, 61.4 c.c. of hydrogen being taken up. On filtering and evaporating to dryness, a crystalline mass was obtained, which could be recrystallised from acetone-alcohol mixture and sublimed readily in the high vacuum at 155-160°, giving long needles of m.p. 181-182°. Yield 0.5 gm.

A sample submitted to analysis gave the following results:

1.620 mg. subst. gave 4.15 mg. CO_2 ... 69.86% C.
1.66 mg. H_2O ... 11.39% H.

1.043 mg. subst. gave 0.047 c.c. N
760 mm., 24.5° 5.17% N.

Calculated for $C_{14}H_{27}O_2N$) .... C, 69.71
) .... H, 11.20
dihydrodesoxy carpamic acid) .... N, 5.81

13. Attempted Halogenation of Carpamic Acid by Hydrobromic Acid.

0.1 Gm. of carpamic acid hydrochloride was heated in a sealed tube with excess of hydrobromic acid (density 1.49) at 160° for 12 hours. The residue obtained on evaporation of hydrobromic acid when recrystallised from alcohol gave a m.p. of 145°.

A/

A second tube was heated at 170° for 12 hours with hydrobromic acid of density 1.7; considerable charring was observed. The residue obtained on evaporation was recrystallised from alcohol, m.p. 145°.

A sample submitted to analysis gave the following results:

20.30 mg. subst. required 0.58 c.c. N/10 $AgNO_3$ = 22.7% Br.

Calculated for $C_{14}H_{28}O_3NBr$ carpamic acid hydrobromide, 23.3% Br.

14. Reduction of Carpamic Acid by Phosphorus and Hydriodic Acid.

Carpamic acid was heated in sealed tubes at various temperatures with different quantities of phosphorus and hydriodic acid. The hydrocarbon was isolated in each case by the same procedure. The product was diluted with water and shaken up with ether. The ether extract was freed from iodine by treatment with powdered copper, washed with dilute aqueous sodium carbonate and with water, dried over sodium sulphate, and the ether evaporated off. The colourless oil so obtained which/

which was insoluble in water but soluble in the usual organic solvents, distilled in the high vacuum at about 90° and could not be crystallised. The best yield was obtained on heating 0.5 gm. of carpamic acid hydrochloride with 0.5 gm. of red phosphorus and 5 gm. of hydriodic acid (density 1.7) at 320-330° for 7 hours. Maximum yield 100 mg.

A sample was purified for analysis by dissolving in ether and shaking up with a crystal of potassium permanganate (to oxidise any incompletely reduced material), washing successively with dilute acid, alkali, and water, and finally distilling carefully in vacuo the oil obtained on evaporation of the ether.

On analysis the following results were obtained:

Sample A.

3.17 mg. gave 10.00 mg. CO_2	86.03%	C
3.93 mg. H_2O	13.87%	H

Sample B.

1.667 mg. gave 5.15 mg. CO_2	84.28%	C
2.10 mg. H_2O	14.10%	H

Calculated results for

$C_{13}H_{28}$	84.8%	C
	15.2%	H
$C_{14}H_{30}$	84.85%	C
	15.15%	H
C_nH_{2n}	85.7%	C
	14.3%	H

As/

As a standard for comparison myristic acid was treated in exactly the same manner as the carpamic acid hydrochloride and the hydrocarbon so obtained was submitted to analysis.

3.495mg. gave 10.840 mg. CO_2 84.59% C
4.845 mg. H_2O 15.51% H

The analysis indicates that the purification was adequate. Apart from the low value for carbon in analysis B, the results indicate that the hydrocarbon has the formula C_nH_{2n} and as the method of preparation precludes the possibility of an unsaturated compound, the presence of a ring is indicated.

Molecular weight determinations by the method of Rast gave the following results:

Mg. subst.	Mg. camphor		Constant	Mol.Wt.
0.432	5.118	17.3°	39.5	192
0.510	6.494	16.8°	39.5	185
Calculated for $C_{14}H_{28}$	.....		.....	196

The refractive index was also determined.

R.I. 1.4448

C-methyl analysis.

Mg. subst.	c.c. N/100 NaOH	$C-CH_3$
6.268	3.15	0.93

15. Action of Hydrogen Peroxide on Carpamic Acid Hydrochloride.

0.38 Gm. of carpamic acid hydrochloride was dissolved in 6 c.c. of water and 3.8 gm. of 3% hydrogen peroxide added slowly during 8 hours, a trace of platinum black being added as catalyst. After standing three days the mixture was worked up. Carpamic acid hydrochloride was recovered in almost quantitative yield.

16. Oxidation of Desoxy Carpamic Acid by Ozone.

0.5 gm. of carpamic acid hydrochloride was converted to desoxy carpamic acid as described in experiment 12. The crude oil freed from inorganic material was dissolved in water and ozonised, the ozone being passed through for several hours. The resulting mixture was heated to destroy any ozone complex which might be present, and then extracted with ether in a continuous extractor for several hours. The ether extract was washed with acid and with water and dried over sodium sulphate.

On/

On evaporation of the ether an oil was obtained which could not be crystallised, distilled in the high vacuum (bath at 130-140°) was insoluble in water and soluble in alkali. The equivalent weight was determined by titration and found to be 184 and 197. The oil was esterified by diazomethane, the resulting ester dissolved in alcohol and allowed to stand with excess of concentrated ammonia (density 880). After several days there were signs of separation of a crystalline product but in such small quantity that nothing definite could be isolated. According to theory, keto decoic acid (equiv. wt. 186) was to be expected.

17. Oxidation of Desoxy Carpamic Acid by Potassium Permanganate.

Desoxy carpamic acid was prepared by treating carpamic acid hydrochloride (0.9 gm.) with phosphorus pentachloride and removing the halogen by boiling with alcoholic potash as in experiment 12. The resulting mixture was made just alkaline to phenolphthalein and treated with 2% aqueous potassium permanganate. 78 c.c. were decolorised in the cold. The flask was then heated on the water/

water bath when a further 72 c.c. of permanganate were required before the mixture retained its pink colour on standing. The precipitated manganese dioxide was filtered off and the solution made acid with hydrochloric acid and extracted with ether in a continuous extractor for 24 hours. The aqueous acid solution [A] was set aside for further examination. The ether extract was washed, dried and the ether distilled off. The resulting oil was sparingly soluble in water and in ether. Further examination indicated the presence of two substances in this oil, one more soluble in ether than the other. The oil was washed with small quantities of ether and the substance obtained on evaporation of the washings distilled in vacuo. The residual fraction [B], which was not taken up into the ether, was set aside for further examination.

Two fractions were collected on distillation:

1. Semi solid oily mass (bath at 170-190°), pressure 1 mm.
2. Solid crystalline mass (bath at 190-210°).

A small residue did not distil.

Fraction 2 /

Fraction 2 was purified by repeated recrystallisation from water. After five recrystallisations the m.p. rose to 103-104°, and further recrystallisation had no further effect. This substance was suspected from the results of Barger, Girardet and Robinson (Helv. Chim. Acta, 1933, 26, 91) to be azelaic acid. A mixed m.p. was therefore carried out with an authentic specimen of azelaic acid supplied by Professor Robinson (synthetic).

Synthetic azelaic acid	m.p. 105-106°
Unknown acid.. ...	m.p. 103-104°
Mixture	m.p. 103-104.5°

There was not sufficient material for an analysis.

The aqueous acid solution A, which was retained from the continuous ether extraction, was further examined. The acid solution was evaporated to dryness and extracted with alcohol. The evaporation and extraction was repeated until all the inorganic material was removed. The residue was distilled/

distilled in a high vacuum. Most of the residue distilled with the metal bath at 150-160°. The distillate was soluble in water, alcohol and acetone and sparingly soluble in ether. No precipitate was obtained with picric acid. The substance was acidic and the residue obtained on evaporation of a portion of the substance with ammonia gave a pyrrole pine splint reaction on distillation with zinc dust. The substance distilled at atmospheric pressure without decomposition, but it could not be crystallised. It is suggested therefore that this substance is a mixture of succinic acid with some other fatty acid produced during oxidation.

The residue B (p. 36) was washed with ether and set aside in a desiccator for some weeks when a few crystals separated. These were insoluble in ether and were acidic, but the quantity available was so small that recrystallisation was found to be impracticable. It is tempting to suggest that this substance is a trace of suberic acid but no real evidence can be put forward. The solubility and the m.p. of the crude crystals (110-124°), however /

however, are in agreement with this assumption.

18. The preparation of N-methyl carpaine.

1 Gram of carpain was allowed to stand at room temperature with excess of methyl iodide for several days, the methyl iodide was then evaporated off and the residue shaken up in the cold with freshly prepared silver oxide in methyl alcohol. After filtration the solution was diluted with water, extracted with ether, the ether evaporated off and the residual N-methyl carpaine recrystallised from methyl ethyl ketone.

m.p. 71°. Yield 0.45 gm.

19. The exhaustive methylation of carpaine.

(a) Using methyl iodide.

0.25 Gm. of N-methyl carpaine was dissolved in excess of methyl iodide, an equal quantity of methyl alcohol added and the mixture refluxed for several hours. The residue left on evaporation of the solvent could be obtained in a crystalline form from methyl alcohol, but a sharp melting point could not be obtained. It was therefore dissolved in methyl/

methyl alcohol and shaken up in the cold with freshly prepared silver oxide. The filtrate was evaporated to dryness and the residue distilled. The metal bath was first raised to 230° at a pressure of 200 mm. Hg., when water was found to distil over; the pressure was then reduced to 15 mm. Hg,when a colourless oil distilled over. This oil was again treated with methyl iodide and silver oxide in the same way, and the residue again distilled. A clear oil distilled over with the bath at 250° under a pressure of 20 mm. and trimethylamine was given off. The oil was dissolved in ether and washed with dilute acid and alkali to remove any non-neutral or water soluble material. The ether solution was dried over sodium sulphate and the ether evaporated off when 8 mg. of a colourless neutral oil was obtained which solidified on standing.

(b) Using ethyl iodide.

N-ethyl carpaine was prepared in the same way as N-methyl carpaine,using ethyl iodide in place of methyl iodide. The exhaustive ethylation of 0.2 gm. of N-ethyl carpaine was carried out in the same manner as the exhaustive methylation and the neutral oil/

oil isolated. Yield 9 mg.

These yields could not be bettered by modification of the experimental conditions, there being apparently reactions other than simple methylation of the nitrogen taking place (cf. van Ryn. Arch. Pharm. 231, 184, 1897).

20. Catalytic reduction of the neutral oil of Experiment 19.

The combined products of the previous experiment (17 mg.) were dissolved in methyl alcohol, a trace of platinum catalyst (Adam's catalyst) added, and the substance reduced with hydrogen. 2 c.c. of hydrogen were adsorbed. On filtration and evaporation of the methyl alcohol a saturated neutral semi-crystalline substance was obtained.
Yield 16 mg.

In this substance the lactone ring of carpaine is presumably intact.

21. Hydrolysis of the lactone ring in the product of Experiment 20.

16 Mg. substance from the previous experiment was allowed to stand with alcoholic potash overnight/

night, the mixture was then diluted with water and extracted with ether to remove any neutral ether soluble material, the solution was then acidified and again extracted with ether, the ether solution washed with water, dried, and the ether evaporated off. The residue was distilled in a high vacuum with the bath at 110°. The distillate was a colourless crystalline mass soluble in ether, alcohol, acetone etc. and in aqueous alkali, but insoluble in water. Yield 9 mg.

A suitable solvent for recrystallisation could not be found and it was therefore decided to prepare the p-phenylphenacyl ester. In this experiment the lactone ring is presumably opened so that the resulting compound will be a hydroxy-fatty acid.

22. Preparation of the p-phenylphenacyl ester of the oxy acid of Experiment 21.

3.5 mg. oxy acid and 4.0 mg. pf p-phenylphenacyl bromide were boiled up with 0.15 c.c. N/10 sodium hydroxide and just sufficient water-alcohol mixture to dissolve the reactants in a small flask fitted with a reflux condenser, for several hours. On cooling and allowing the mixture to stand in air so that the alcohol evaporated slowly, a white more

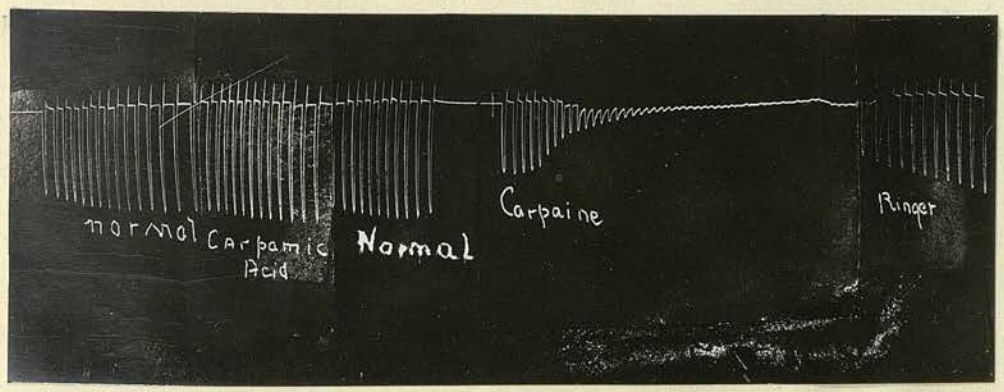
or/

more or less crystalline substance separated. The mixture was centrifuged and the supernatant liquid run off. The residue was dissolved in alcohol-water mixture, filtered, and allowed to stand, when a small quantity of colourless crystalline material separated. This substance was isolated in the same way as before and again recrystallised. The final product (0.25 mg.) appeared to be quite pure, having a sharp melting point (91°), but owing to the small quantity available and to the difficulty of obtaining the p-phenylphenacyl esters of the higher fatty acids in a perfectly pure state, this melting point must at present be accepted with some reserve.

23. A comparison of the physiological action of carpamic acid and of carpaine on the isolated frog's heart.

According to van Ryn (loc. cit.) carpaine has a powerful depressor action on the heart. It was considered of interest to compare the action of carpamic acid with that of carpaine. For this purpose a frog's heart was isolated and perfused with Ringer's solution, a tracing of the heart beat being/

being recorded. The carpamic acid hydrochloride was dissolved in Ringer's solution in a concentration of 1 mg.% and perfused through the heart without any appreciable effect on the beat. Finally the heart was perfused with Ringer's solution containing an equivalent concentration of carpaine hydrochloride, when a marked depression of the beat was observed, leading finally to a complete stoppage of the beat in diastole. The rate of the heart beat was not affected. The beat returned to normal on washing out the heart with fresh Ringer's solution.



Tracing showing the effect of carpaine and of carpamic acid on the isolated frog's heart.

III. SUMMARY.

III. SUMMARY.

The constitution of the alkaloid carpaine is studied and a structure suggested containing a ten membered lactone ring. A possible method of synthesis in the plant is indicated, and a comparison of the heart depressor action of carpaine and carpamic acid is made.